

A rapid and simplified method for quantitative analysis by thin-layer chromatography

Cylindrical thin-layer chromatography

In quantitative evaluations by the usual thin-layer chromatographic techniques, the sample solution has to be applied with a capillary pipette in a streak in order to expect good separation. The capillary pipette and ordinary thin-layer plate usually used, however, are not suitable for quantitative assay since the capillary pipette disturbs the surface of the thin layer, resulting in uneven solvent flow and unsatisfactory separation, thus causing errors. Furthermore, the use of the pipette often causes the removal of a portion of the sample together with the adsorbent from the layer so that a large amount (ml grade) of sample solution cannot be applied to the thin layer on the plate.

Several devices have been described for overcoming these difficulties¹⁻⁶. This paper deals with a new simple apparatus for use in quantitative assays by which the application of a large amount of sample solution (ml grade) is possible without disturbing the surface of the thin layer.

Apparatus

The apparatus, depicted in Fig. 1, consists of: (1) an inner cylinder which is like a test tube, the bottom of which has a small hole (about 1 mm in diameter) that is used for exuding the sample solution and developing solvent from the inside to the outside; and (2) an outer cylindrical cover which is used as the developing chamber and has an inlet and outlet that are used for introducing gas while applying the sample solution.

Operation

Preparation of thin-layer cylinder

45-50 g of adsorbent is suspended in 100 ml of chloroform-methanol (2:1, v/v) as in PELFER's method⁷. A homogeneous slurry is immediately prepared by stirring the suspension with a glass rod or by shaking the suspension in a glass-stoppered flask.

The inner cylinder described above is dipped into the homogeneous slurry, the hole in the cylinder being stoppered with a glass rod the end of which is covered with a rubber tube (as depicted in Fig. 2). The cylinder is slowly withdrawn from the slurry and a cylinder the outside of which is freshly coated with a thin layer of adsorbent is obtained. The wet cylinder is left standing in the atmosphere to dry. The solvent quickly evaporates. The thin-layer cylinder thus prepared is sufficiently stable and is ready for use. The dried thin layer on the cylinder may be exposed to steam for a short time and dried at 105° for about an hour for activation.

Application of sample solution

The thin-layer cylinder is put into the outer cover-cylinder. A sample solution can be applied to the thin-layer cylinder (see Fig. 3) by two different methods. One of them is the "total volume method" and the other the "partial volume method".

Total volume method. The sample solution (V_p ml) is pipetted into the thin-layer cylinder with a pipette of ml grade such as is used in ordinary volumetric analysis.

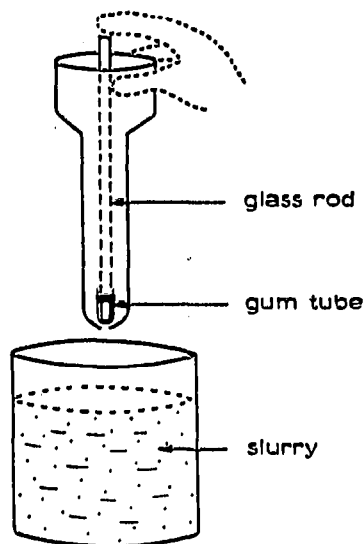
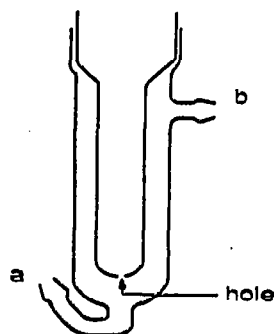


Fig. 1. Apparatus for cylindrical thin-layer chromatography. a = Inlet; b = outlet.

Fig. 2. Preparation of thin-layer cylinder.

During the course of this procedure, a flow of gas (e.g., N_2 , H_2 , CO_2) is introduced through the inlet a or the air is sucked through from the outlet b by a water aspirator. After almost all the sample solution in the thin-layer cylinder has exuded from inside to the outer thin layer through the small hole, a little solvent is poured into the thin-layer cylinder and allowed to stand for a few minutes to wash out the rest of the sample solution in the cylinder to the outer thin layer. This washing procedure (pour-

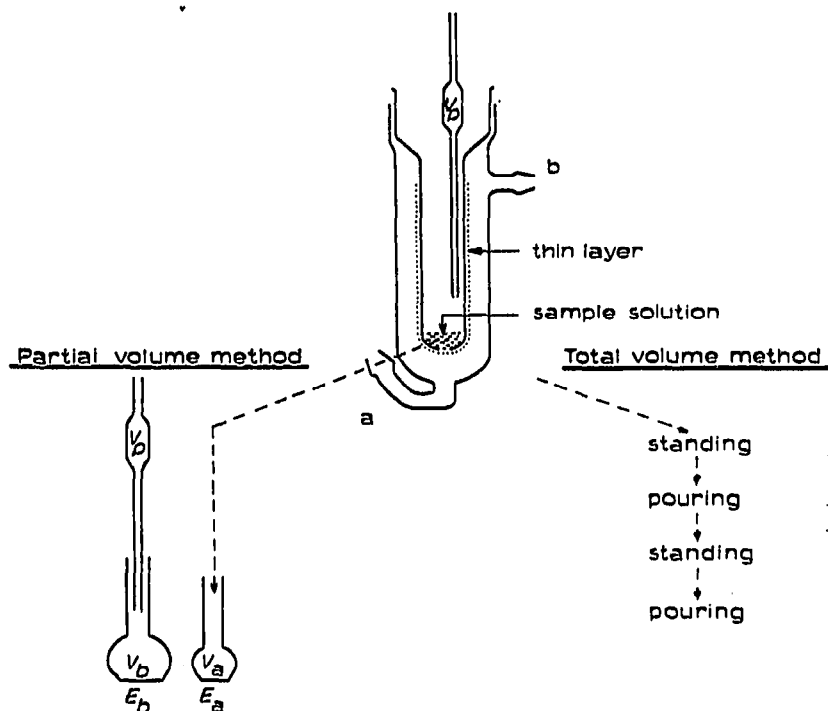


Fig. 3. Application of sample solution. a = Inlet; b = outlet.

ing and standing) is repeated until the whole sample solution in the cylinder has completely exuded to the outer thin layer.

Partial volume method. The sample solution (V_p ml) is pipetted into the thin-layer cylinder as described above and left standing for a suitable time. After an optional amount of the sample solution has exuded to the outer thin layer, the rest* of the sample solution in the cylinder is removed into a volumetric flask (V_a ml) and the cylinder is washed with solvent. The removed sample solution and washings are combined and the whole made up to V_a ml with the solvent (V_a solution). V_p ml of the original sample solution is pipetted into a volumetric flask (V_b ml) and the whole made up to V_b ml with the solvent (V_b solution). The optical densities of the V_a and V_b solutions are determined at a suitable** wave length giving optical densities E_a and E_b , respectively. In this case it is advisable to adjust the volumes V_a and V_b so that E_a and E_b are nearly equal***.

The volume (V) of the sample solution exuded to the outer thin layer is calculated from the following formula:

$$V \text{ (ml)} = \frac{(1 - E_a \times V_a)}{E_b \times V_b} \times V_p$$

Development

After the application of the sample solution to the thin layer as described above, the gas flow is stopped, and a developing solvent is poured into the bottom of the outer cover-cylinder through the inlet a and the outlet b is stoppered. The developing solvent is then poured into the thin-layer cylinder (Fig. 4). The time of development

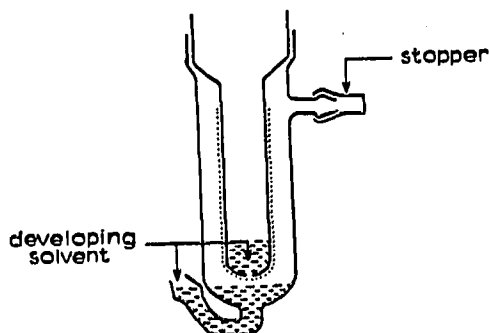


Fig. 4. Development.

depends on the size of the hole in the cylinder. After development, the thin-layer cylinder is dried in the air or in an inert gas and examined under ultraviolet light or visualised by other detection methods (*e.g.*, iodine vapor).

The band in the form of a ring, detected on the cylindrical thin layer, is scraped off with a flat spatula while rotating the cylinder and the sample is extracted with a suitable solvent. The substance in the extract is then measured quantitatively.

* If the sample solution contains an internal standard as in the case of a technique similar to quantitative gas chromatography, it is not necessary to measure the volume of the remaining sample solution.

** It is advisable that the absorption curve of the sample solution should be nearly flat at the wave length chosen.

*** Because it is possible that the substance may have a different molecular absorption at different dilutions.

Conclusions

The merits of applying this apparatus and technique to quantitative analysis are:

(1) A large amount of a sample solution can be applied without any particular skill and without disturbing the thin layer.

(2) The partial volume method can be adopted.

(3) The surface of the thin layer is not disturbed at all since the sample solution automatically exudes to the thin layer through a small hole covered with the thin layer.

(4) The solvent in the sample solution can be evaporated off in an atmosphere of inert gas. This is especially suitable for applying a substance which is stable in the state of a solution but labile in the absence of a solvent.

(5) Since the thin layer is cylindrical, there is no "edge effect"⁸ (an abnormal increase of R_F values in the areas close to the sides of the chromatoplate) like there is with an ordinary plate.

(6) Since the chromatogram is cylindrical, it is easier to scrape off the adsorbate from the cylinder by rotating it than from the usual flat plate.

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